EXHIBIT J

N-nitroso compounds and cancer incidence: the European Prospective Investigation into Cancer and Nutrition (EPIC)–Norfolk Study[1–3]

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ABSTRACT

Background: Humans are exposed to preformed *N*-nitroso compounds (NOCs) and endogenous NOCs. Several NOCs are potential human carcinogens, including *N*-nitrosodimethylamine (NDMA), but evidence from population studies is inconsistent.

Objective: We examined the relation between dietary NOCs (NDMA), the endogenous NOC index, and dietary nitrite and cancer incidence in the European Prospective Investigation into Cancer and Nutrition (EPIC)–Norfolk, United Kingdom, study.

Design: This was a prospective study of 23,363 men and women, aged 40–79 y, who were recruited in 1993–1997 and followed up to 2008. The baseline diet was assessed with food-frequency questionnaires.

Results: There were 3268 incident cancers after a mean follow-up of 11.4 y. Dietary NDMA intake was significantly associated with increased cancer risk in men and women [hazard ratio (HR): 1.14; 95% CI: 1.03, 1.27; *P* for trend = 0.03] and in men (HR: 1.24; 95% CI: 1.07, 1.44; *P* for trend = 0.005) when the highest quartile was compared with the lowest quartile in age- and sex-adjusted analyses but not in multivariate analyses (HR: 1.10; 95% CI: 0.97, 1.24; HR for men: 1.18; 95% CI: 1.00, 1.40; *P* for trend $\geq$ 0.05). When continuously analyzed, NDMA was associated with increased risk of gastrointestinal cancers (HR: 1.13; 95% CI: 1.00, 1.28), specifically of rectal cancer (HR: 1.46; 95% CI: 1.16, 1.84) per 1-SD increase after adjustment for age, sex, body mass index, cigarette smoking status, alcohol intake, energy intake, physical activity, education, and menopausal status (in women). The endogenous NOC index and dietary nitrite were not significantly associated with cancer risk. There was a significant interaction between plasma vitamin C concentrations and dietary NDMA intake on cancer incidence (*P* for interaction $<$ 0.00001).

Conclusions: Dietary NOC (NDMA) was associated with a higher gastrointestinal cancer incidence, specifically of rectal cancer. Plasma vitamin C may modify the relation between NDMA exposure and cancer risk. *Am J Clin Nutr* 2011;93:1053–61.

INTRODUCTION

N-nitroso compounds (NOCs) can induce cancer in about 40 different animal species, including higher primates, and are carcinogenic in multiple organs in animals (1). In humans, there is supporting evidence for a role of NOCs in the cause of certain cancers such as gastric, esophageal, nasopharyngeal, and colon cancers (2, 3). Several NOCs are potential human carcinogens (4, 5), including *N*-nitrosodimethylamine (NDMA), which has been classified as "probably carcinogenic to humans" (4, 6).

Human beings are exposed to preformed NOCs from sources such as tobacco products, diet, drugs, and occupational environments and NOCs produced in vivo (3). Endogenously, NOCs that comprise nitrosamines and nitrosamides can be generated when nitrite reacts with the degradation products of amino acids in the stomach. An estimated 45–75% of the total NOC exposure is contributed by endogenous synthesis (7). Hence, most studies have included nitrite when examining the relation between NOCs and cancer risk. Attempts have been made to obtain direct estimations of endogenous NOCs via urine- and fecal-output chemical analyses. However, these methods are not feasible in large epidemiologic studies. Thus, one study determined an endogenous NOC exposure index (ENOC) on the basis of several human controlled-diet studies (8). These studies have presented evidence for an increased NOC production in the colon [measured as apparent total NOCs (ATNCs)] with red-meat consumption, which is possibly because of haem, and in a dose-response manner with increasing red-meat intake (9–12).

With consideration of the NOC exposure routes and sites, studies that investigated NOCs and cancer have mainly focused on cancers related to the gastrointestinal tract including gastric, esophageal, and colorectal cancers. However, the evidence from findings has not been consistent and conclusive. Some of these studies also investigated the possible effect modification by dietary and plasma vitamin C concentrations because the nitrosation reaction can be inhibited by the presence of antioxidants such as vitamins C and E (13, 14).

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Overall, human exposure to NOCs and subsequent cancer risk has not been studied extensively. In this study, we examined the relation between dietary NOCs (measured by NDMA), endogenous NOCs (ENOC), and dietary nitrite, and cancer incidence in the European Prospective Investigation into Cancer and Nutrition (EPIC)–Norfolk study. We also investigated if there was any evidence for an effect modification of these exposures by plasma vitamin C concentrations in relation to cancer incidence.

SUBJECTS AND METHODS

Study population

The EPIC-Norfolk study is a prospective cohort of 25,639 men and women, aged 40–79 y, who were recruited between 1993 and 1997 in Norfolk, United Kingdom. The procedures of the EPIC-Norfolk study and details of participant recruitment have been previously described (15). Ethical approval for the study was obtained from the Norfolk and Norwich Hospital Ethics Committee. After excluding 1445 participants with cancer at baseline, 2 participants without confirmation of a cancer-diagnosis date, and 829 participants with missing dietary NDMA, ENOC, and dietary nitrite data from food-frequency questionnaires (FFQs), a total of 23,363 participants were available for analyses.

Endpoint ascertainment

Incident cases of cancer [International Classification of Diseases, 10th revision, codes: C00–C97 excluding C44 (nonmelanoma skin cancer)] were identified up to 30 June 2008 by the East Anglian Cancer Registry. Participants were flagged with the National Health Service Central Register for death and cancer incidence as well as with the East Anglian Cancer Registry. For the purpose of this study, the follow-up for each participant began at the date of the baseline health examination. A total of 3268 incident cancer cases were identified.

Data collection

In brief, participants completed consent forms and a detailed health and lifestyle questionnaire. The questionnaire included questions on cigarette smoking, alcohol consumption, physical activity, social class, occupational history, use of medication, history of disease, short family history of major diseases, and reproductive history for women. Participants who consented attended a health examination. Trained nurses collected weights, heights, waist and hip circumferences, respiratory functions, and blood pressure data by using standard protocols.

Body mass index (BMI) was calculated by weight in kilograms divided by height in meters squared (kg/m^2). Current cigarette smoking status was categorized into 3 groups (never, former, and current), physical activity status was categorized into 4 groups (active, moderately active, moderately inactive, and inactive), and educational level was categorized into 4 groups (none or primary, secondary O level, secondary A level, and degree). The O level indicates the completion of schooling to the age of 15 y, and the A level to indicates the completion of schooling to the age of 17 y. For women, menopausal status (pre- and postmenopausal) and use of postmenopausal hormone therapy (never, former, and current) were also included in the analyses.

Nonfasting blood samples were collected in plain and citrate bottles. A detailed description of the storage method has been previously described (16). For plasma vitamin C, plasma was stabilized with 10% metaphosphoric acid, and samples were then stored at $-70°C$ until analyses were carried out in ≤ 1 wk of sampling. The plasma ascorbic acid concentration (μmol ascorbic acid/L) was estimated with a fluorometric assay (17, 18).

Dietary data

EPIC-Norfolk uses the following 3 different methods to record the participants' diet: the 24-h recall, FFQ, and 7-d food diary (19). For this study, data for dietary NDMA, ENOC, and dietary nitrite were only available for the validated FFQ method (20). Participants who returned signed consent forms were sent the FFQ before the health check. Completed FFQs were returned during the baseline health examination; trained nurses checked and completed the FFQs when necessary. The semiquantitative EPIC-FFQ was designed to establish the average consumption of foods during the preceding year (21). It consists of a food list with 131 questionnaire lines. Participants were requested to assess the consumption frequency of each food item on the basis of standard portion sizes. The frequency of consumption was based on a set of 9 frequency choices for consumption that ranged from never or less than once a month to ≥ 6 times/d. The Compositional Analyses from Frequency Estimates program (EPIC-Norfolk) then calculated nutrients on the basis of the FFQ data entered. Dietary data obtained for this study included intakes of alcohol (g alcohol/d), energy (kcal/d), and vitamin C (mg vitamin C/d).

Dietary NDMA (μg NDMA/d, presented in ng), ENOC (μg/d), and dietary nitrite (mg nitrite/d) data were obtained from the EPIC-EURGAST study (8). NDMA and nitrite consumptions were estimated by matching FFQ food items with a food database of potential carcinogens (8, 22). This extensive database was compiled by conducting a literature search (1980–2003) to identify sources of data on the concentrations of nitrosamines in foods. The database was reviewed by experts on both the specific chemical compounds and the food-composition databases (22). Country-specific analyzed values were chosen when available to closely match the NDMA content of foods in the respective countries. For food items without analyzed values available, the value of the nearest comparable food was assigned. An average of all available suitable values was assigned when several matches for a food item were available. NDMA was estimated for the EURGAST study because there was considerably more information for NDMA than for other nitrosamines shown in foods. For the endogenous NOC exposure, an index (the ENOC) was previously determined (8) by using the estimated iron content from meat intake and fecal ATNC formation from several human controlled-diet studies (9–12). Dietary iron values from meat for diets administered in the studies were estimated on the basis of the UK Food Composition database. Afterwards, the correlation between iron intake from meat and fecal ATNC levels was assessed, and the ENOC was predicted by using the linear regression accordingly (8).

Statistical analyses

Descriptive statistics, including means and percentages, were used to show the distribution of baseline characteristics in cases

and noncases. These were formally compared by using the *t* test for continuous variables and the chi-square test for categorical variables. There were some extreme outliers for NDMA, ENOC, and nitrite. We dealt with these in a number of ways. For quartile analyses, these values were included in the top quartile and were left unchanged. For continuous analyses, we log transformed the values. However, because log-transformed values are difficult to interpret, and findings were essentially unchanged, results for the continuous analyses without log transformation were shown. To avoid having a disproportionate effect of extreme outliers when these were used as continuous variables, outliers of NDMA (4.4% of the study population), ENOC (0.7% of the study population), and nitrite (0.1% of the study population) were recoded as a maximum of the mean ± 4 SD of each exposure. A Cox proportional hazards regression model was used to examine the relation between cancer incidence and dietary intake of NDMA, ENOC, and dietary nitrite. These 3 exposures were analyzed as continuous variables (per approximate 1-SD increase) and as categorical variables (quartiles). Hazard ratios were measured with corresponding 95% CIs. Initial models were adjusted for age and sex. The multivariate model was additionally adjusted for BMI, cigarette smoking status, alcohol intake, energy intake, physical activity status, educational level, and menopausal status (in women). BMI, alcohol intake, and energy intake were analyzed as continuous variables, whereas the rest of the covariates were analyzed as categorical variables. Two other models were further adjusted for plasma vitamin C concentrations and vitamin C intakes separately. A single multiplicative interaction term was used to assess the potential interactive effects between NDMA, nitrite, and ENOC and plasma vitamin C concentrations on cancer risk, and its significance was tested by using the likelihood ratio test. Plasma vitamin C is a more accurate biomarker than is dietary vitamin C, which has dietary measurement-error issues. Plasma vitamin C was analyzed as a continuous variable and as categorical variable (cutoff of 50 μmol/L, which was the rounded-up mean value for this study population).

For the specific cancer-site analyses, cases were defined on the basis of the primary cancer diagnosed. We investigated cancers associated with the gastrointestinal tract and also common cancers in this study population. These cancers (International Classification of Diseases codes) included esophageal (C15), stomach (C16), colon (C18), rectum (C19–C20), breast (C50), prostate (C61), lung (C71), and ovarian (C56–C57) cancers. The rest of cancers in the study population were categorized as others. Cancers of the esophagus, stomach, colon, and rectum were grouped under gastrointestinal cancers. Individuals with missing values were excluded from specific analyses. All tests for significance were 2-sided, and *P* values were considered statistically significant at the 5% level. Statistical analyses were performed with STATA statistical software (version 10.1; StataCorp, College Station, TX).

RESULTS

There were data available for analyses on 23,363 participants (10,783 men and 12,580 women) with a mean age at recruitment of 58.6 y. After a mean follow-up of 11.4 y, 3268 individuals (1671 men and 1597 women) were identified with incident cancer.

Descriptive characteristics of cancer cases and noncases are presented in **Table 1**. Cancer cases were older, heavier, and more likely to be men and less active than were noncases. Greater proportions of cases had none or a primary level of education and were current or former smokers. In women, cases were more likely to be postmenopausal. Dietary intakes of both NDMA and ENOC were significantly higher in cases than in noncases. Plasma vitamin C concentrations were significantly lower in cases than noncases. **Table 2** shows selected means and proportions for characteristics of participants by NDMA quartile.

Hazard ratios (HRs) for cancer incidence associated with dietary NDMA, ENOC, and dietary nitrite exposures by quartiles in the population and also in men and women separately are shown in **Table 3**. Individuals in the highest quartile of NDMA intake, compared with those in the lowest quartile of NDMA intake, had significant elevated cancer risk (HR: 1.14; 95% CI: 1.03, 1.27; *P* for trend = 0.03) after adjustment for age and sex but not in the multivariate adjustment. When men and women were analyzed separately, this association remained significant only in men (HR: 1.24; 95% CI: 1.07, 1.44; *P* for trend = 0.005) but not in women. There was no significant association in cancer risk across quartiles for dietary nitrite and ENOC. When exposure variables were analyzed as continuous variables, NDMA intake was associated with increased risk in the age- and sex-adjusted and multivariate analyses except for the analyses that adjusted for plasma vitamin C concentrations (**Table 4**). Although risk estimates observed in men and women were in the same direction, only risk estimates for men were significant in all analyses. In the case of ENOC, an increased HR was only observed in men in age-adjusted analyses but not in multivariate analyses.

HRs of cancer risk at specific cancer sites are shown in **Table 5**. There was an overall increased cancer risk with NDMA when cancers of the gastrointestinal tract were analyzed as combined. Two categories, rectal cancer and other cancers showed increased risks with significant HRs of 1.46 and 1.11 (for an increment of ≈0.05 μg NDMA), respectively. The 5 cancers listed in the order of highest incidence in the latter category were malignant melanoma of skin, malignant neoplasms of bladder, corpus uteri, pancreas, and kidney (except for renal pelvis) cancers.

We also tested for potential interactions between plasma vitamin C concentrations and the 3 exposures of interest. There were significant interactive effects between plasma vitamin C concentrations and dietary NDMA intake on cancer risk in simple age- and sex-adjusted and multivariate models. Similar findings were observed for ENOC but only in the age- and sex-adjusted model (*P* for interaction = 0.009). There was no evidence for any interaction in the case of nitrite. The effect of plasma vitamin C concentrations and dietary NDMA exposure on cancer risk is shown in **Table 6**. When analyzed categorically at the cutoff plasma vitamin C concentration of 50 μmol/L, the HR per unit increase in dietary NDMA exposure was higher in individuals with plasma vitamin C concentrations $<$50 μmol/L than in those with plasma vitamin C concentrations $\geq$50 μmol/L in both models.

DISCUSSION

In this large prospective study of dietary NOCs in relation to cancer risk, we showed that gastrointestinal cancer incidence was associated with dietary NDMA but not with ENOC and nitrite. To the best of our knowledge, no other study has investigated all-cancer incidence and exposure to both endogenous and exogenous dietary NOC in a prospective population study.

TABLE 1
Descriptive characteristics of cancer cases and noncases[1]

Characteristics	Cases	Noncases	*P*
Age at baseline (y)	62.4 ± 8.6[2]	58.0 ± 9.3	<0.0001
No. of subjects	3268	20,095	
BMI (kg/m^2)	26.6 ± 3.9	26.3 ± 3.9	<0.0001
No. of subjects	3260	20,059	—
Sex [*n* (%)]			
M	1671 (51.1)	9112 (45.3)	<0.001
F	1597 (48.9)	10,983 (54.7)	—
Total no. of subjects	3268	20,095	—
Cigarette smoking status [*n* (%)]			
Never	1296 (40.1)	9406 (47.2)	<0.001
Former	1505 (46.5)	8277 (41.5)	—
Current	434 (13.4)	2250 (11.3)	—
Total no. of subjects	3235	19,933	—
Physical activity status [*n* (%)]			
Active	525 (16.1)	3798 (18.9)	<0.001
Moderately active	685 (21.0)	4619 (23.0)	—
Moderately inactive	892 (27.3)	5812 (28.9)	—
Inactive	1166 (35.7)	5865 (29.2)	—
Total no. of subjects	3268	20,094	—
Educational level [*n* (%)]			
None/primary	1295 (39.7)	7189 (35.8)	<0.001
Secondary O level	302 (9.2)	2098 (10.4)	—
Secondary A level	1316 (40.3)	8128 (40.5)	—
Degree	352 (10.8)	2669 (13.3)	—
Total no. of subjects	3265	20,084	—
Menopausal status (women) [*n* (%)]			
Premenopausal	230 (14.4)	2610 (23.8)	<0.001
Postmenopausal	1364 (85.6)	8365 (76.2)	—
Total no. of subjects	1594	10,975	—
Hormone replacement therapy use (women) [*n* (%)]			
Never	1108 (69.5)	7415 (67.6)	0.29
Former	173 (10.8)	1253 (11.4)	—
Current	313 (19.6)	2307 (21.0)	—
Total no. of subjects	1594	10,975	—
NDMA (ng/d)	59.1 ± 48.5	57.0 ± 47.2	0.02
No. of subjects	3268	20,095	—
ENOC (μg/d)	73.1 ± 19.5	72.3 ± 19.2	0.04
No. of subjects	3268	20,095	—
Nitrite (mg/d)	1.49 ± 0.50	1.48 ± 0.51	0.10
No. of subjects	3268	20,095	—
Energy (kcal/d)	2052 ± 586	2033 ± 580	0.08
No. of subjects	3236	19,924	—
Alcohol (g/d)	8.9 ± 13.5	8.7 ± 12.9	0.37
No. of subjects	3236	19,924	—
Vitamin C (mg/d)	123.6 ± 59.8	124.6 ± 58.6	0.37
No. of subjects	3236	19,924	—
Plasma vitamin C (μmol/L)	51.3 ± 21.3	53.7 ± 20.0	<0.00001
No. of subjects	2867	17,697	—

[1] NDMA, *N*-nitrosodimethylamine; ENOC, endogenous *N*-nitroso compound exposure index. Percentages may not sum to 100% because of rounding. *P* values are for the significance of the *t* test for continuous variables and of the chi-square test for categorical variables.
[2] Mean ± SD (all such values).

We investigated all cancers because NOCs have been shown to be carcinogenic in multiple organs in animals (1).

When 532 gastrointestinal cancer cases were analyzed collectively, we showed a significant positive association with NDMA intake. On the basis of the digestive system route, the esophagus, stomach, colon, and rectum were logically the sites that were susceptible to exogenous NOCs. Moreover, NDMA has been shown to induce DNA adduct formation in human colonocytes (23, 24). This association was not seen for nitrite and ENOC, but we hypothesized significant findings more likely at specific sites shown to support endogenous nitrosation that involved these 2 exposures (ie, the stomach and large bowel) (25). Nitrite reacts with nitrosatable compounds in the stomach, and the large bowel is a rich source of nitrogenous residues where bacterial flora might catalyze nitrosation reactions. We examined gastrointestinal cancer sites separately. There was no significant

TABLE 2
Selected characteristics of participants by *N*-nitrosodimethylamine (NDMA) quartile (Q)[1]

	NDMA intake (ng/d)			
Characteristics	Q1	Q2	Q3	Q4
Intake of NDMA (n = 23,363) (ng/d)[2]	16.8	33.4	53.1	125.9
Age at baseline (n = 23,363) (y)[2]	59.7	58.8	58.7	57.3
BMI (n = 23,319) (kg/m^2)[2]	26.2	26.2	26.3	26.5
Male sex (n = 10,783) (%)	25.2	32.6	46.8	80.1
Cigarette smoking status (n = 23,168) (%)				
Never	53.2	51.5	46.7	33.4
Former	35.9	37.8	41.9	53.3
Current	10.9	10.8	11.4	13.3
Physical activity status (n = 23,362) (%)				
Active	15.3	16.5	18.6	23.6
Moderately active	21.6	23.6	22.6	23.1
Moderately inactive	29.5	29.5	29.9	25.9
Inactive	33.7	30.4	29.0	27.3
Educational level (n = 23,349) (%)				
None/primary	42.9	39.0	35.1	28.3
Secondary O level	9.8	10.8	10.6	10.0
Secondary A level	37.0	38.3	40.8	45.7
Degree	10.2	11.9	13.6	16.0
Menopausal status in women (n = 12,569) (%)				
Postmenopausal	81.1	77.5	75.1	69.4
Hormone replacement therapy use in women (n = 12,569) (%)				
Never	66.3	67.6	69.6	69.4
Former	12.3	11.8	9.9	10.2
Current	21.4	20.6	20.5	20.4
ENOC (μg/d) (n = 23,363)[2]	64.4	71.0	76.0	78.3
Nitrite (mg/d) (n = 23,363)[2]	1.17	1.41	1.63	1.69
Energy (kcal/d) (n = 23,160)[2]	1746	1959	2148	2286
Alcohol (g/d) (n = 23,160)[2]	3.0	4.7	7.0	20.0
Vitamin C (mg/d) (n = 23,160)[2]	125.7	126.0	127.1	118.8
Plasma vitamin C (μmol/L) (n = 20,564)[2]	56.4	54.8	52.9	49.6

[1] ENOC, endogenous *N*-nitroso compound exposure index. Percentages may not sum to 100% because of rounding.
[2] Values are means.

association with esophageal and stomach cancers for all 3 exposures. A systematic review (26) of the epidemiologic evidence for nitrosamine (an NOC) and nitrite and gastric and esophageal cancer risks showed insufficient evidence for esophageal cancer but a positive association between nitrite and nitrosamine intake and gastric cancer. In this review, cohort studies reported no association for nitrite (27) and NDMA (23) intakes with gastric cancer risk. Similar to our study, the EURGAST study reported that ENOC was positively but not significantly associated with gastric cancer risk (8). Nevertheless, ENOC was significantly associated with noncardia cancer risk but not with cardia cancer. The review showed 3 case-control studies that reported a significant positive association for nitrite intake and, similarly, 3 studies for nitrosamine intake and gastric cancer risk (26). We acknowledge the limitation of the small number of cases for esophageal and stomach cancers in our study.

Furthermore, in this study, only rectal cancer, but not colon cancer, was significantly associated with NDMA exposure. The elevated risk in rectal cancer with NDMA intake was also observed for dietary nitrite and ENOC exposures, but the associations were not significant. Previous studies on meat consumption and colorectal cancer have reported a stronger positive association for rectal cancer than for colon cancer (28, 29). Possible reasons for this variation include the greater contact time with potential carcinogens, including NOCs, and higher fecal matter concentrations are toward the rectum rather than the colon. A Finnish study reported an increased colorectal cancer risk with NDMA exposure in 73 cases (23). When we combined colon and rectal cancer cases (n = 413) in our study, the relative risk estimate was >1.0 but not significant. No significant associations were shown for breast, prostate, lung, and ovarian cancers.

Vitamin C may protect against carcinogenesis by inhibiting the nitrosation process and, thus, reducing endogenous nitrosation formation (13). Two gastric cancer case-control studies examined vitamin C effect modification and showed cancer risk most pronounced in people who had high nitrite and low vitamin C intakes (30, 31). However, these studies did not account for the potential effect modification by *Helicobacter pylori*, which is an important risk factor for gastric cancer. A gastric cancer case-control study that considered *H. pylori*–infection status observed interactions between plasma vitamin C concentrations and ENOC (8). Our study also tested for the potential interaction between the 3 exposures of interest and plasma vitamin C concentrations. There were significant interactive effects between plasma vitamin C concentrations and dietary NDMA intake on cancer risk in age- and sex-adjusted and multivariate models. Similar findings were observed for ENOC but only in the age- and sex-adjusted model (P for interaction = 0.009) (data

TABLE 3
Dietary *N*-nitrosodimethylamine (NDMA), endogenous *N*-nitroso compound exposure index (ENOC), and dietary nitrite exposure in relation to cancer risk[1]

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
All (*n* = 23,363)					
NDMA					
n/*N* (%)	787/5054 (13.5)	815/5026 (14.0)	812/5029 (13.9)	854/4986 (14.6)	—
HR (95% CI)[2]	1.00	1.07 (0.97, 1.18)	1.05 (0.95, 1.16)	1.14 (1.03, 1.27)	0.03
HR (95% CI)[3]	1.00	1.06 (0.96, 1.18)	1.03 (0.93, 1.15)	1.10 (0.97, 1.24)	0.22
ENOC					
n/*N* (%)	779/5062 (13.3)	843/4998 (14.4)	828/5013 (14.2)	818/5022 (14.0)	—
HR (95% CI)[2]	1.00	1.04 (0.95, 1.15)	1.01 (0.91, 1.11)	0.99 (0.90, 1.10)	0.73
HR (95% CI)[3]	1.00	1.02 (0.92, 1.12)	0.97 (0.88, 1.07)	0.95 (0.85, 1.05)	0.19
Nitrite					
n/*N* (%)	778/5063 (13.3)	821/5020 (14.1)	806/5035 (13.8)	863/4977 (14.8)	—
HR (95% CI)[2]	1.00	1.01 (0.92, 1.12)	0.95 (0.86, 1.05)	1.01 (0.92, 1.11)	0.89
HR (95% CI)[3]	1.00	1.02 (0.92, 1.13)	0.96 (0.86, 1.07)	1.02 (0.90, 1.14)	0.91
Men (*n* = 10,783)					
NDMA					
n/*N* (%)	231/1243 (15.7)	311/1591 (16.4)	424/2307 (15.5)	705/3971 (15.1)	—
HR (95% CI)[2]	1.00	1.12 (0.94, 1.33)	1.11 (0.95, 1.31)	1.24 (1.07, 1.44)	0.005
HR (95% CI)[3]	1.00	1.12 (0.94, 1.33)	1.10 (0.93, 1.29)	1.18 (1.00, 1.40)	0.08
ENOC					
n/*N* (%)	338/2006 (14.4)	438/2286 (16.1)	418/2329 (15.2)	477/2491 (16.1)	—
HR (95% CI)[2]	1.00	1.09 (0.94, 1.25)	1.01 (0.87, 1.17)	1.10 (0.96, 1.26)	0.37
HR (95% CI)[3]	1.00	1.05 (0.91, 1.21)	0.95 (0.82, 1.10)	1.04 (0.90, 1.20)	0.95
Nitrite					
n/*N* (%)	356/2028 (14.9)	403/2184 (15.6)	421/2326 (15.3)	491/2574 (16.0)	—
HR (95% CI)[2]	1.00	0.98 (0.85, 1.13)	0.94 (0.81, 1.08)	1.00 (0.87, 1.15)	0.95
HR (95% CI)[3]	1.00	0.98 (0.85, 1.14)	0.93 (0.80, 1.09)	0.98 (0.83, 1.16)	0.75
Women (*n* = 12,580)					
NDMA					
n/*N* (%)	556/3811 (12.7)	504/3435 (12.8)	388/2722 (12.5)	149/1015 (12.8)	—
HR (95% CI)[2]	1.00	1.04 (0.92, 1.17)	1.02 (0.90, 1.16)	1.12 (0.93, 1.34)	0.33
HR (95% CI)[3]	1.00	1.03 (0.91, 1.17)	0.99 (0.86, 1.14)	1.05 (0.86, 1.29)	0.83
ENOC					
n/*N* (%)	441/3056 (12.6)	405/2712 (13.0)	410/2684 (13.2)	341/2531 (11.9)	—
HR (95% CI)[2]	1.00	1.01 (0.88, 1.16)	1.02 (0.89, 1.16)	0.90 (0.78, 1.04)	0.21
HR (95% CI)[3]	1.00	0.99 (0.86, 1.13)	0.99 (0.87, 1.14)	0.85 (0.73, 0.99)	0.06
Nitrite					
n/*N* (%)	422/3035 (12.2)	418/2836 (12.8)	385/2709 (12.4)	372/2403 (13.4)	—
HR (95% CI)[2]	1.00	1.04 (0.91, 1.19)	0.97 (0.85, 1.12)	1.04 (0.90, 1.20)	0.83
HR (95% CI)[3]	1.00	1.06 (0.92, 1.22)	0.98 (0.84, 1.14)	1.05 (0.89, 1.25)	0.83

[1] *n*, number of cases; *N*, total number of participants in the respective quartile of the population; HR, hazard ratio. Individuals with missing values were not included in the analyses.
[2] Cox proportional regression adjusted for age and sex.
[3] Cox proportional regression adjusted for age, sex, BMI, cigarette smoking status, alcohol intake, energy intake, physical activity status, educational level, and menopausal status (in women).

not shown). We did not investigate possible interactions in individual cancers because of the small number of cases. We further examined risk of cancer for dietary NDMA stratified by plasma vitamin C concentrations. The positive association with NDMA was present only in subjects with low plasma vitamin C concentrations. This exploratory finding needs to be confirmed in additional studies.

The mean ENOC (73 μg/d) of cases in our study was lower than the EPIC-Europe estimate (93 μg/d) (8), probably because of the variation of levels across countries. However, similar to the EURGAST study (8), this endogenous index was far greater than the estimated dietary NOC exposure as measured by NDMA intake (≈0.06 μg NDMA/d) in this study. This was in agreement with controlled-feeding studies of high-meat diets that have shown that fecal ATNCs are most likely to have been produced endogenously (9–12).

Several methods, including measuring urinary *N*-nitrosoproline and ATNCs, have been used to attempt to measure the extent of endogenous nitrosation. However, the wide range of possible NOCs that can be formed and the rapid metabolism of NOCs in the body pose challenges to these methods (25). Despite an indirect measure of the endogenous nitrosation of NOCs, the ENOC is a feasible tool for large epidemiologic studies such as the current study. This method estimated the ENOC on the basis of the iron content from meat intakes, and it has previously been shown that the correlation between iron and ENOC was very high (8).

TABLE 4
Dietary *N*-nitrosodimethylamine (NDMA), endogenous *N*-nitroso compound exposure index (ENOC), and dietary nitrite exposure in relation to cancer risk by sex[1]

	Cases/total population	NDMA (per SD)	*P*	ENOC (per SD)	*P*	Nitrite (per SD)	*P*
All							
Adjusted for age and sex	3268/23,363	1.07 (1.03, 1.11)	0.001	1.02 (0.98, 1.05)	0.37	0.99 (0.96, 1.03)	0.62
Multivariate adjustment[2]	3193/22,920	1.07 (1.01, 1.12)	0.01	1.00 (0.96, 1.04)	0.96	0.99 (0.95, 1.03)	0.64
Multivariate adjustment[3]	2808/20,211	1.05 (0.99, 1.11)	0.10	1.00 (0.96, 1.04)	0.97	0.98 (0.94, 1.03)	0.42
Multivariate adjustment[4]	3193/22,920	1.06 (1.01, 1.12)	0.02	1.00 (0.96, 1.04)	0.97	1.00 (0.95, 1.04)	0.84
Men							
Adjusted for age	1671/10,783	1.08 (1.04, 1.13)	<0.001	1.05 (1.00, 1.10)	0.03	0.99 (0.95, 1.04)	0.76
Multivariate adjustment[2]	1636/10,611	1.09 (1.03, 1.16)	0.004	1.03 (0.98, 1.09)	0.22	0.98 (0.93, 1.04)	0.56
Multivariate adjustment[3]	1440/9434	1.07 (1.00, 1.14)	0.04	1.02 (0.97, 1.08)	0.45	0.98 (0.92, 1.04)	0.49
Multivariate adjustment[4]	1636/10,611	1.09 (1.03, 1.16)	0.005	1.03 (0.98, 1.09)	0.22	1.00 (0.93, 1.06)	0.90
Women							
Adjusted for age	1597/12,580	1.09 (1.00, 1.18)	0.05	0.98 (0.93, 1.03)	0.49	1.00 (0.95, 1.05)	1.00
Multivariate adjustment[2]	1557/12,309	1.05 (0.96, 1.16)	0.30	0.96 (0.91, 1.02)	0.19	1.00 (0.94, 1.07)	1.00
Multivariate adjustment[3]	1368/10,777	1.04 (0.94, 1.16)	0.44	0.98 (0.92, 1.03)	0.41	0.99 (0.92, 1.06)	0.69
Multivariate adjustment[4]	1557/12,309	1.05 (0.96, 1.16)	0.29	0.96 (0.91, 1.02)	0.19	1.00 (0.93, 1.07)	0.93

[1] Values are hazard ratios (HRs); 95% CIs in parentheses. Individuals with missing values were not included in the analyses.
[2] Cox proportional regression adjusted for age, sex, BMI, cigarette smoking status, alcohol intake, energy intake, physical activity status, educational level, and menopausal status (in women).
[3] Cox proportional regression adjusted for age, sex, BMI, cigarette smoking status, alcohol intake, energy intake, physical activity status, educational level, menopausal status (in women), and plasma vitamin C concentrations.
[4] Cox proportional regression adjusted for age, sex, BMI, cigarette smoking status, alcohol intake, energy intake, physical activity status, educational level, menopausal status (in women), and vitamin C intake.

The estimated mean intake of NDMA in our study was relatively low compared with that in other studies (0.08–0.55 μg NDMA/d) (32). However, dietary assessment methods were quite different across studies. Moreover, the lack of food-content information is one of the main difficulties in the assessment of effects of dietary nitrosamines (22). NDMA was estimated to examine dietary preformed NOC exposure because it is one of the major dietary nitrosamines, and considerably more information is available for NDMA than for other volatile NOCs in foods. The unavailability of a complete food-composition table for the NOC content in foods (22) probably led to the use of individual food sources as a proxy measure in previous studies (33). These foods may be sources of other compounds that may play a role in cancer risk. Also, a dietary measurement error could arise because of FFQs or other assessment methods used in various studies. The number of food items assessed in these studies ranged from 28 to 474 items (32).

The positive association between NDMA exposure and cancer risk was observed in men but not in women. The NDMA intake was much higher in men (≈0.08 μg NDMA/d) than in women (≈0.04 μg NDMA/d) with a difference of 50%. Other studies have reported differences of 20% and up to a 3 times higher NDMA intake in men (32, 34–36). Perhaps the exposure level in women was not high enough to reach the biological level that could exert carcinogenic effects. The main dietary sources of NDMA include cured meat and beer (37). For example, the

TABLE 5
Dietary *N*-nitrosodimethylamine (NDMA), endogenous *N*-nitroso compound exposure index (ENOC), and dietary nitrite exposure in relation to site-specific cancer risk[1]

Cancer site	*n* (cases)[1]	NDMA (per SD)	*P*	ENOC (per SD)	*P*	Nitrite (per SD)	*P*
Esophageal	55	1.13 (0.77, 1.68)[2]	0.53	1.14 (0.88, 1.48)	0.33	1.14 (0.84, 1.54)	0.39
Stomach	64	1.13 (0.81, 1.57)	0.47	1.13 (0.88, 1.45)	0.34	0.86 (0.63, 1.19)	0.37
Colon	276	0.99 (0.83, 1.18)	0.93	0.95 (0.84, 1.09)	0.49	0.89 (0.77, 1.04)	0.15
Rectum	137	1.46 (1.16, 1.84)	0.001	1.05 (0.88, 1.25)	0.60	1.18 (0.97, 1.44)	0.10
Gastrointestinal	532	1.13 (1.00, 1.28)	0.04	1.02 (0.93, 1.12)	0.66	0.99 (0.89, 1.10)	0.83
Breast	423	1.01 (0.84, 1.20)	0.94	1.03 (0.93, 1.14)	0.60	1.08 (0.96, 1.22)	0.22
Prostate	461	1.01 (0.90, 1.13)	0.92	0.93 (0.84, 1.03)	0.15	0.90 (0.81, 1.01)	0.08
Lung	235	1.05 (0.88, 1.24)	0.60	1.01 (0.89, 1.16)	0.84	0.97 (0.83, 1.14)	0.74
Ovarian	80	0.96 (0.60, 1.53)	0.85	0.84 (0.65, 1.08)	0.18	0.79 (0.58, 1.07)	0.12
Others	1462	1.11 (1.03, 1.19)	0.007	1.02 (0.96, 1.07)	0.57	1.02 (0.95, 1.08)	0.63

[1] Total population = 22,920 individuals.
[2] Hazard ratio; 95% CI in parentheses (all such values). Cox proportional regression adjusted for age, sex, BMI, cigarette smoking status, alcohol intake, energy intake, physical activity status, educational level, and menopausal status (in women).

TABLE 6
Effect of plasma vitamin C concentrations and dietary *N*-nitrosodimethylamine exposure (per SD) on cancer risk[1]

Plasma vitamin C[2]	Cases	Noncases	HR (95% CI)[3]	Multivariate HR (95% CI)[4]
	%	%		
<50 μmol/L	1295 (15.7)	6,934 (84.3)	1.10 (1.04, 1.16)	1.08 (1.00, 1.16)
≥50 μmol/L	1572 (12.7)	10,763 (87.3)	1.02 (0.96, 1.09)	1.01 (0.94, 1.10)
P for interaction	—	—	0.03	0.04
P for interaction (continuous)	2867 (13.9)	17,697 (86.1)	<0.00001	0.006

[1] HR, hazard ratio. Percentages may not sum to 100% because of rounding.
[2] On the basis of the cutoff of the rounded-up mean value.
[3] Cox proportional regression adjusted for age and sex.
[4] Cox proportional regression adjusted for age, sex, BMI, cigarette smoking status, alcohol intake, energy intake, physical activity status, educational level, and menopausal status (in women). Cases and noncases for <50-μmol/L plasma vitamin C concentrations consisted of 1264 and 6805 subjects, respectively; cases and noncases for ≥50-μmol/L plasma vitamin C concentrations consisted of 1544 and 10,598 subjects, respectively.

NDMA content of bacon is ≈0.05–0.16 μg NDMA/100 g bacon (38, 39). The difference in intake could possibly be explained by the fact that men consume these foods more often than women do. Another possibility, given the observed interaction with vitamin C, is that women with higher vitamin C concentrations than men, which, given the interactions, may attenuate a relation between NOCs and cancer.

This study had both strengths and limitations. Potential limitations included the measurement error associated with dietary assessment by using the FFQ method as well as limitations in the estimation of dietary NOCs. However, a random measurement error was only likely to attenuate any real associations but not produce spurious associations. Because this was a prospective study, a dietary assessment was conducted before the diagnosis of cancer, which minimized the recall bias and reverse causation. We also acknowledged the different causes of cancer at different sites.

In conclusion, our results suggested that there was a positive association between NDMA intake and gastrointestinal cancer risk, specifically of rectal cancer. There was evidence that plasma vitamin C concentrations had an effect-modifier role between cancer risk and NDMA exposure. This may explain variations in findings in different populations depending on the prevailing vitamin C exposure.

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